

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph at section [0011] of the published application with the paragraph set forth below.

In one embodiment ~~an~~ a CLL cell line of malignant origin is provided that is not established by immortalisation with EBV. The cell line, which was derived from primary CLL cells, and is deposited under ATCC accession no. PTA-3920. In a preferred embodiment, the cell line is CLL-AAT. CLL-AAT is B-CLL cell line, derived from a B-CLL primary cell.

Please replace the paragraph at section [0033] of the published application with the paragraph set forth below:

FIG. 12. shows the identification of scFv antigens by immunoprecipitation and mass spectrometry. CLL-AAT cells were labeled with a solution of 0.5 mg/ml sulfo-NHS-LC-biotin (Pierce) in PBS, pH 8.0 for 30'. After extensive washing with PBS to remove unreacted biotin, the cells were disrupted by nitrogen cavitation and the microsomal fraction was isolated by differential centrifugation. The microsomal fraction was resuspended in NP40 Lysis Buffer and extensively precleared with normal rabbit serum and ~~protein A-sepharose~~ Protein A SEPHAROSE®. Antigens were immunoprecipitated with HA-tagged scFv antibodies coupled to Rat Anti-HA agarose beads (Roche). Following immunoprecipitation, antigens were separated by SDS-PAGE and detected by Western blot using streptavidin-alkaline phosphatase (AP) or by Coomassie G-250 staining. ScFv-7, an antibody which doesn't bind to CLL-AAT cells, was used as a negative control. Antigen bands were excised from the Coomassie-stained gel and identified by mass spectrometry (MS). MALDI-MS was performed at the Proteomics Core Facility of The Scripps Research Institute (La Jolla, Calif). μ LC/MS/MS was performed at the Harvard Microchemistry Facility (Cambridge, Mass.).

Please replace the paragraph at section [0091] of the published application with the paragraph set forth below:

Peripheral blood from a patient diagnosed with CLL was obtained. The WBC count was 1.6×10^8 /ml. Mononuclear cells were isolated by ~~Histopaque-1077~~ HISTOPAQUE®-1077 density gradient centrifugation (Sigma Diagnostics, St. Louis, Mo.). Cells were washed twice with Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), and resuspended in 5 ml of ice-cold IMDM/10% FBS. Viable cells were counted by staining with trypan blue. Cells were mixed with an equal volume of 85% FBS/15% DMSO and frozen in 1 ml aliquots for storage in liquid nitrogen.

Please replace the paragraph at section [0101] of the published application with the paragraph set forth below:

The libraries were enriched for CLL cell surface-specific antibodies by positive-negative selection with a magnetically-activated cell sorter (MACS) as described by Siegel et al. (1997, J. Immunol. Methods 206:73-85). Briefly, phagemid particles from the scFv antibody library were preincubated in MPBS (2% nonfat dry milk, 0.02% sodium azide in PBS, pH 7.4) for 1 hour at 25° C. to block nonspecific binding sites. Approximately 10^7 primary CLL cells were labeled with mouse anti-CD5 IgG and mouse anti-CD19 IgG conjugated to paramagnetic microbeads (Miltenyi Biotec, Sunnyvale, Calif.). Unbound microbeads were removed by washing. The labeled CLL cells ("target cells") were mixed with an excess of "antigen-negative absorber cells", pelleted, and resuspended in 50 μ l (10^{10} - 10^{11} cfu) of phage particles. The absorber cells serve to soak up phage that stick non-specifically to cell surfaces as well as phage specific for "common" antigens present on both the target and absorber cells. The absorber cells used were either TF-1 cells (a human erythroleukemia cell line) or normal human B cells isolated from peripheral blood by immunomagnetic negative selection (~~StemSep~~ StemSep™ system, StemCell Technologies, Vancouver, Canada). The ratio of absorber cells to target cells was approximately 10-fold by volume. After a 30 minute incubation at 25° C., the cell/phage mixture was transferred to a

~~MiniMACS~~ MiniMACS™ MS⁺ separation column. The column was washed twice with 0.5 ml of MPBS, and once with 0.5 ml of PBS to remove the unbound phage and absorber cells. The target cells were eluted from the column in 1 ml of PBS and pelleted in a microcentrifuge at maximum speed for 15 seconds. The captured phage particles were eluted by resuspending the target cells in 200 µl of acid elution buffer (0.1 N HCl, pH adjusted to 2.2 with glycine, plus 1 µg/ml BSA). After a 10 minute incubation at 25° C., the buffer was neutralized with 12 µL of 2M Tris base, pH10.5, and the eluted phage were amplified in E. coli for the next round of panning. For each round of panning, the input and output phage titers were determined. The input titer is the number of reamplified phage particles added to the target cell/absorber cell mixture and the output titer is the number of captured phage eluted from the target cells. An enrichment factor (E) is calculated using the formula $E = (R_n \text{ output} / R_n \text{ input}) / (R_1 \text{ output} / R_1 \text{ input})$. In most cases, an enrichment factor of 10^2 - 10^3 fold should be attained by the third or fourth round.

Please replace the paragraph at section [0108] of the published application with the paragraph set forth below:

To screen individual scFv clones following panning, TOP10F' cells were infected with phage pools as described above, spread onto LB plates containing carbenicillin and tetracycline, and incubated overnight at 37° C. Individual colonies were inoculated into deep 96-well plates containing 0.6-1.0 ml of SB-carbenicillin medium per well. The cultures were grown for 6-8 hours in a ~~HiGro~~ HiGro® shaking incubator (GeneMachines, San Carlos, Calif.) at 520 rpm and 37° C. At this point, a 90 µl aliquot from each well was transferred to a deep 96-well plate containing 10 µL of DMSO. This replica plate was stored at -80° C. IPTG was added to the original plate to a final concentration of 1 mM and shaking was continued for 3 hours. The plates were centrifuged at 3000xg for 15 minutes. The supernatants containing soluble scFv antibodies were transferred to another deep 96-well plate and stored at -20° C.

Please replace the paragraph at section [0126] of the published application with the paragraph set forth below:

293-EBNA cells (Invitrogen) were seeded at 2.5×10^6 per 100 mm dish. 24 hours later the cells were transiently transfected using ~~Polyfect~~ PolyFect® reagent (QIAGEN) according to the manufacturer's instructions. Cells were cotransfected with 7.2 µg of OX-2/CD200 cDNA in vector pCEP4 (Invitrogen) and 0.8 µg of ~~pAdVantage~~ pAdVantage™ vector (Promega). As a negative control, cells were cotransfected with empty pCEP4 vector plus ~~pAdVantage~~ pAdVantage™. 48 hours after transfection, approximately 90% of the cells expressed OX-2/CD200 on their surface as determined by flow cytometry with the scFv-9 antibody.

Please replace the paragraph at section [0137] of the published application with the paragraph set forth below:

Cytokines such as IL-2, IFN-γ, IL-4, IL-10 and IL-6 found in the tissue culture supernatant were quantified using ELISA. Matched capture and detection antibody pairs for each cytokine were obtained from R+D Systems (Minneapolis, Minn.), and a standard curve for each cytokine was produced using recombinant human cytokine. Anti-cytokine capture antibody was coated on the plate in PBS at the optimum concentration. After overnight incubation, the plates were washed and blocked for 1 hour with PBS containing 1% BSA and 5% sucrose. After 3 washes with PBS containing 0.05% ~~Tween~~-TWEEN®, supernatants were added at dilutions of two-fold or ten-fold in PBS containing 1% BSA. Captured cytokines were detected with the appropriate biotinylated anti-cytokine antibody followed by the addition of alkaline phosphatase conjugated streptavidin and SigmaS substrate. Color development was assessed with an ELISA plate reader (Molecular Devices).